Fetal Infection of the Baboon (*Papio cynocephalus*) with Vaccinia Virus (37461)

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In utero infection of a fetus as a result of maternal involvement with one or another virus has been well documented (1–12). What has not been determined, however, is the spectrum of viral involvement and relationship to the period of gestation. Furthermore, specific localization of any given virus within the tissues of the fetus, following infection, is still relatively unknown. Studies to date have failed to relate maternal infection to the process of morphogenesis and embryogenesis in primates.

The purpose of this report is to provide information on the passage of vaccinia virus across the placenta at different stages of gestation. This virus was selected because of its size, known involvement in production of disease in primates, and well-studied biologic characteristics. Choice of the baboon (*Papio cynocephalus*) as the experimental model was based upon previous studies (13, 14) demonstrating its susceptibility to vaccinia virus as well as its close similarity to man in fetal morphogenesis and placental ultrastructure (15–17).

Materials and Methods. Animals. Nine pregnant baboons were divided into three groups depending upon their stage of pregnancy; first, second, or third trimester. No attempt was made, at this time, to define the precise stages of gestation as we were primarily concerned with attempts to determine localization of virus in the placenta and fetal tissues. The placenta and fetus were removed by cesarean section in the usual manner at the desired stage of pregnancy. Pre-inoculation bloods were obtained in all instances and the animals were observed for evidence of clinical disease.

Virology. The IHD strain of vaccinia virus with a titer of $10^{6.3}$ $TCID_{50}$ ml in Vero cells was given intravenously in 2.0 ml amounts. Ability to produce a lesion was determined by intradermal inoculation of the test animal. One animal (0269) developed a lesion at the site of intravenous inoculation. Following virus administration, all animals were routinely sampled (throat and rectal swabs and blood) for evidence of virus shedding and seroconversions. Placentas and fetuses were removed 2, 4, and 7 days post-inoculation.

Histology. Appropriate fetal and placental tissues were fixed in Bouin's, Carnoy's and buffered 10% neutral formalin. The specimens were paraffin embedded, sectioned and stained in Masson's trichome stain and hematoxylin-eosin.

Virus isolation. Coincident with collection of samples for histological examination, similar materals were obtained for virus isolation. Pieces of tissue were inoculated directly onto monolayers of primary baboon kidney cells (BKC) or seeded for explant culture (18). Media from explant cultures were tested on BKC for the presence of virus. Tissues from second and third trimester fetuses were tested by both techniques, first trimester fetuses by explant only.

Results. Table I provides data obtained from examination of tissues from the indicated animals. It will be noted that vaccinia virus was recovered, generally from the throat of the mothers, *i.e.*, from 5 of 8 animals. The 3 animals not yielding virus were all at term. One of the 5 baboons shedding virus from their throats was also found to have virus in its stool. Virus isolations from the fetal tissues were less frequent (3 of 8 animals) and

TABLE I. Virus Isolation from Maternal and Fetal Specimens.

Animal no.	Stage of pregnancy when inoculated	Virus isolation: Maternal HI antibody Pre	Maternal HI antibody Post	Maternal Throat	Maternal Rectal	Fetal Tissue	Fetal Days post inoculation[c]
4037	1st Trimester	<10	320	2, 9[a]	—[b]	Liver, skin and muscle	2
0896	1st Trimester	<10	80	7	—	Skin and muscle amniotic membrane	7
0269	2nd Trimester	<10	80	4	—	—	4
0561	2nd Trimester	<10	40	7	7	Spleen	7
3947	3rd Trimester	<10	40	2	—	—	2
0609	3rd Trimester	<10	320	—	—	—	2
0471	3rd Trimester	<10	320	—	—	—	4
0641	3rd Trimester	<10	40	—	—	—	7
0229	3rd Trimester	<10	320	—	7	—	5[d]

[a] Day after inoculation on which isolation was made.
[b] No virus isolated.
[c] When cesarean section was performed.
[d] Day after inoculation when baby was born.

were mainly from skin and muscle. Virus was also found in liver, spleen and amniotic membrane, although such tissues as kidney, spleen, adrenal, bladder, lung, thymus, heart, brain, testis, foreskin, umbilical cord, and placenta were examined.

Examination of maternal sera by hemagglutination-inhibition tests indicated that all of the mothers had been infected and seroconverted.

Histological examination of the tissues at the time periods indicated failed to show any histological lesions that may be associated with vaccinia virus.

Discussion. The results obtained here clearly demonstrate that vaccinia virus was transferred across the placenta at early and, in one instance, at midterm pregnancy. Additional studies are necessary to ascertain the precise periods of time that permit viral passage from mother to fetus. Failure to detect virus in the placenta by the procedures employed may be due to the time lapse between inoculation and sampling, *i.e.*, 2–7 days. Obviously other complementary studies are necessary to determine this point. These results are in accord with the finding that vaccinia virus may affect the human fetus as a result of infection (19). We have previously demonstrated that the poxviruses are capable of producing infection of this species of baboon (13).

Neff *et al.* (20) point out that even though the risks of congenital malformations from vaccinia are small, vaccination during pregnancy should be carefully considered prior to administration. All our fetuses at time of section were normal in appearance and alive. One newly born animal from a mother shedding virus (No. 0229) died three days after birth (5 days after inoculation) but virus was not recovered from any of its tissues.

These studies, while preliminary in nature, suggest that the animal model used here may provide an opportunity for expanded investigations with this and other viruses at different stages of pregnancy (especially as determined by accurately timed pregnancies), perhaps even use of other simian species. Such studies would permit the collection of carefully controlled data subject to detailed analysis on mother, fetus, and placenta in their reactions to viral infection.

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